Review

Signaling pathways in diabetic nephropathy

Daiji Kawanami, Keiichiro Matoba and Kazunori Utsunomiya

Division of Diabetes, Metabolism and Endocrinology, Department of Internal
Medicine, Jikei University, School of Medicine, Minato-ku, Tokyo, Japan

Summary. Diabetic nephropathy (DN) is a major cause
of end-stage renal disease (ESRD), however, specific
treatment for DN has not yet been elucidated. Therefore,
it is critically important to understand the molecular
mechanism underlying DN to develop cause-related
therapeutic strategy. To date, various factors such as
hemodynamic changes and metabolic pathways have
been shown to be involved in the pathogenesis of DN.
Excessive glucose influx activates cellular signaling
pathways, including the diacylglycerol (DAG)-protein
kinase C (PKC) pathway, advanced glycation end-
products (AGE), polyol pathway, hexosamine pathway
and oxidative stress. These factors interact with one
another, thereby facilitating inflammatory processes,
leading to the development of glomerulosclerosis under
diabetic conditions. In addition to metabolic pathways,
Rho-kinase, an effector of small-GTPase binding protein
Rho, has been implicated as an important factor in the
pathogenesis of DN. A number of studies have
demonstrated that Rho-kinase plays key roles in the
development of DN by inducing endothelial dysfunction,
mesangial excessive extracellular matrix (ECM)
production, podocyte abnormality, and tubulointerstitial
fibrosis. In this review article, we describe our current
understanding of the signaling pathways in DN.

Key words: Diabetic nephropathy, Hemodynamic
mechanism, Metabolic pathways, Rho-kinase

Offprint requests to: Daiji Kawanami, Division of Diabetes, Metabolism
and Endocrinology, Department of Internal Medicine, Jikei University
School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461,
Japan. e-mail: daijika@jikei.ac.jp

Introduction

The numbers of patients with diabetes are increasing
in both developed and developing countries. The factor
which determines the prognosis of diabetic patients is
diabetic complications, in particular, diabetic
nephropathy (DN). Great advances have been made in
developing novel anti-diabetic agents in the last decade.
However, overcoming diabetic complications has not
been accomplished. This fact indicates that conventional
therapy to manage risk factors such as hyperglycemia,
hypertension, and dyslipidemia is not sufficient to inhibit
the development and progression of DN.

It is widely accepted that DN is strongly associated
with cardiovascular events. There is clear association
between the progression of DN and risk of death (Adler
et al., 2003). Therefore, understanding the molecular
mechanism underlying DN is critically important not
only for scientific reasons, but also to improve the
prognosis of patients with diabetes. In this review article,
we describe the signaling pathways involved in the
development of DN. Furthermore, a significant body of
evidence demonstrates that small GTPase binding
protein Rho and its effector Rho-kinase play key roles in
the pathogenesis of DN. According to this background,
the emerging role of Rho-kinase in DN is also discussed.

Pathogenesis of DN

Hemodynamic mechanism

Glomerular pressure is constantly maintained by
balancing the vascular resistance of afferent arterioles
and efferent arterioles and autoregulation, such as
tubuloglomerular feedback (TGF) (Premaratne et al.,

2015). However, these balances are impaired under diabetic conditions. Hyperglycemia increases the blood flow into afferent arterioles, resulting in an increase in glomerular filtration (glomerular hyperfiltration) and excess glomerular pressure (intraglomerular hypertension). Glomerular hyperfiltration is a compensatory mechanism that covers a reduced number of functioning nephrons and maintains the glomerular filtration rate of the kidney by increasing the filtration rate per single nephron (Helal et al., 2012). However, cellular functions collapse under chronic glomerular hyperfiltration and sustained intraglomerular hypertension.

Intrarenal RAS activity is increased in the diabetic milieu. Angiotensin II is locally produced in the kidney and contracts efferent arterioles, leading to intraglomerular hypertension (Forbes et al., 2007). Hyperglycemia and insulin resistance cause RAS activation. A recent study demonstrated that angiotensin-converting enzyme (ACE)2 plays a key role in the development of glomerular hyperfiltration (Tikellis et al., 2014). ACE2-deficient mice showed a lack of glomerular hyperfiltration or increased glomerular hydrostatic capillary pressure in response to hyperglycemia or a high-protein diet (Tikellis et al., 2014). Clinically, RAS inhibitors have been shown to attenuate albuminuria in patients with diabetes (Brenner et al., 2001; Makino et al., 2007). Angiotensin II has fibrotic effects on the glomeruli. It has been shown that angiotensin II stimulates TGF-β-mediated extracellular matrix (ECM) production in mesangial cells (Kagami et al., 1994) by activating protein kinase C (PKC) (Arendshorst et al., 1999; Nagahama et al., 2000) and NF-κB (Ruiz-Ortega et al., 2000).

Regulation of contraction of afferent arterioles is impaired in diabetes, therefore the blood flow into the glomeruli is increased. Calcium influx into the vascular smooth muscle of the afferent arterioles is reduced and their contraction is suppressed (Carmines et al., 1996). In addition, it has been reported that the increase of prostaglandin is associated with impaired myogenic responsiveness of the afferent arterioles (Hayashi et al., 1992).

Finally, TGF plays an important role in auto-regulation of the afferent arterioles (Gilbert, 2014). The macula densa controls contraction and dilatation of the afferent arterioles by the concentration of NaCl. In response to an increased NaCl concentration in the macula densa, the afferent arterioles contract to reduce the blood flow into the glomeruli. Conversely, a decreased NaCl concentration in the macula densa leads to afferent arteriole dilatation to increase the blood flow into the glomeruli to maintain a constant GFR (De Nicola et al., 2014). In diabetes, the glucose concentration in the glomerular filtrate is increased and reabsorption of glucose through sodium glucose co-transporter (SGLT) 2 in the proximal tubule is enhanced. In addition, reabsorption of sodium in the proximal tubule is also enhanced by SGLT2. SGLT2 inhibitors have recently appeared as a novel therapeutic option against diabetes. As SGLT2 inhibitors suppress reabsorption of sodium along with glucose, they may contract the afferent arterioles by inducing TGF (Gilbert, 2014). According to these observations, the preventive effects of SGLT2 inhibitors on glomerular hyperfiltration have been proposed. Indeed, recent clinical studies demonstrated that SGLT2 inhibitors suppress glomerular hyperfiltration (Cherney et al., 2014) and attenuate albuminuria in patients with diabetes (Yale et al., 2014).

Metabolic mechanisms

Although intracellular glucose is metabolized primarily by glycolysis, excess intracellular glucose is subject to metabolism by alternative pathways under diabetic conditions. The accumulation of metabolites of these pathways plays an important role in the pathogenesis of DN. The diacylglycerol (DAG)-PKC pathway, advanced glycation end-products (AGE), polyol pathway, hexosamine pathway and oxidative stress are known (Fig. 1).

DAG-PKC pathway

PKC is a serine/threonine-related protein kinase and is involved in various cellular signaling pathways (Geraldes and King, 2010). PKC has been shown to be activated in the vessels, retina and glomeruli under diabetic conditions (Rask-Madsen and King, 2013). PKC has multiple isoforms that are classified into conventional PKCs (α, β, and γ), novel PKCs (δ, ε, η, and θ), and atypical PKCs (ι and ζ) according to their structures and modes of activation (Geraldes and King, 2010; Rask-Madsen and King, 2013). Conventional isoforms are activated by Ca^{2+}, DAG, phospholipids, such as phosphatidylserine and phorbol esters. Novel
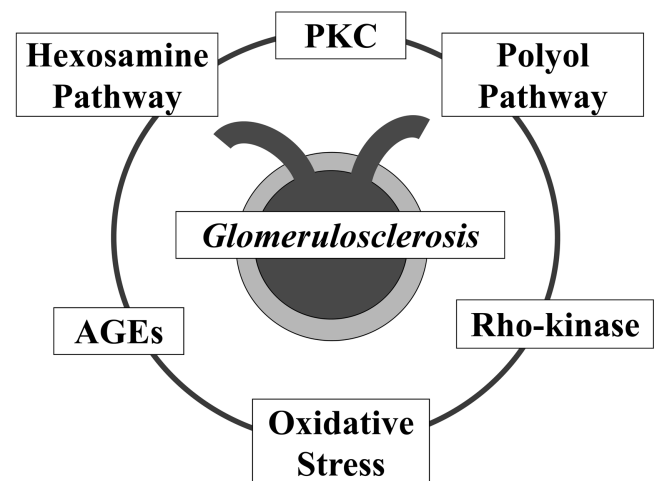

Fig. 1. Signaling pathways in DN. Multiple metabolic pathways are activated under diabetic conditions. These pathways interact with one another, thereby promoting the development of DN.

isoforms are activated by DAG, but not Ca^{2+}, because they do not possess Ca^{2+} binding activity. Atypical isoforms do not contain either Ca^{2+} binding activity or DAG binding activity. The total DAG levels are elevated in vascular tissues and glomeruli under diabetic conditions (Craven et al., 1990). DAG levels are also elevated in the diabetic milieu due to an increase in the glycolytic intermediate dihydroxyacetone phosphate. This intermediate is reduced to glycerol-3-phosphate, which subsequently increases *de novo* synthesis of DAG (Xia et al., 1994; Geraldes and King, 2010). Accumulated DAG in the cell activates PKC by binding to the DAG binding site in the regulatory domain of PKC (Newton, 2001). AGEs and oxidative stress also increase PKCβ activity in mesangial cells (Scivittaro et al., 2000). PKC activates downstream inflammatory signals, including oxidative stress, thereby promoting the progression of DN.

Genetic deletion and pharmacologic inhibition of PKCβ has been shown to exhibit beneficial effects against DN. Ruboxistaurin, a PKCβ inhibitor, has been shown to inhibit DN in an experimental diabetes model by improving hemodynamics (Ishii et al., 1996) and ameliorating mesangial expansion (Koya et al., 2000). Consistent with these findings, a study utilizing PKCβ knockout mice showed attenuation of albuminuria and glomerulosclerosis in a streptozotocin-induced diabetes model accompanied by inhibition of NADPH oxidases and fibrotic factors, such as endothelin (ET)-1, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β and connective tissue growth factor (CTGF) (Ohshiro et al., 2006). PKCβ has been shown to be involved in regulation of glucagon-like peptide (GLP)-1 receptors in glomerular endothelial cells (Mima et al., 2012). Endothelial cell-specific overexpression of PKCβ resulted in a greater loss of the endothelial GLP-1 receptor expression. Furthermore, albuminuria and mesangial expansion were exaggerated in these mice under diabetic conditions (Mima et al., 2012). It has been shown that PKCα knockout mice are resistant to the development of albuminuria and glomerular hyperfiltration (Menne et al., 2004). Furthermore, dual inhibition of PKCα and PKCβ showed a significant reduction in albuminuria and ECM production in experimental diabetic models (Menne et al., 2013).

Advanced Glycation End-products (AGEs)

AGEs such as carboxymethyl lysine are products of nonenzymatic glycation and protein oxidation. AGE formation is accelerated by hyperglycemia (Ramasamy et al., 2011). AGEs alter the protein function and elicit inflammatory signaling pathways via receptor for AGEs (RAGE). AGEs have been shown to promote glomerulosclerosis and podocyte abnormalities via NF-ϰB activation, nitric oxide (NO) downregulation, and ROS generation (Vlassara et al., 1994; Lu et al., 1998; Checcherita et al., 2016). RAGE has been shown to interact with non-AGE ligands such as S100/calgranulins and high mobility group box 1 (HMGB1)

(Hofmann et al., 1999; Taguchi et al., 2000). Targeting RAGE by gene-deletion or neutralizing antibody resulted in attenuation of glomerulosclerosis and podocyte abnormalities in experimental diabetic models (Wendt et al., 2003; Flyvbjerg et al., 2004; Jensen et al., 2006; Reiniger et al., 2010).

Polyol pathway/Hexosamine pathway

Glucose is reduced to glucose-6-phosphate by glucokinase and then proceeds to the TCA cycle in the glycolytic pathway. Under hyperglycemic conditions, alternative pathways such as the polyol and hexosamine pathways are utilized. In the polyol pathway, glucose is reduced to sorbitol by aldose reductase (AR) and subsequently converted to fructose by sorbitol dehydrogenase (SDH). The accumulation of sorbitol has been implicated in the pathogenesis of diabetic complications. Increased intracellular glucose promotes the flux of glucose into the polyol pathway. This pathway consumes NAD^+ in sorbitol reductase reaction and NADPH in AR reaction as a coenzyme. NADH is produced in sorbitol reductase reaction, thereby increasing the intracellular $NADH/NAD^+$ ratio, leading to inhibition of glyceraldehyde-3 phosphate dehydrogenase (GAPDH) activity (Forbes et al., 2007). Because GAPDH is required for the glycolytic conversion of glyceraldehyde 3-phosphate, inactivation of GAPDH results in an increase of glyceraldehyde 3-phosphate, which causes production of methylglyoxal, an AGE precursor and DAG (Brownlee, 2001; Rask-Madsen and King, 2013). Furthermore, a decrease of NADPH causes inhibition of NO synthase (NOS) activity because NOS utilizes NADPH as a coenzyme (Forstermann and Sessa, 2012). It has been shown that AR inhibition attenuates high glucose-mediated PKC activation and the TGF-β expression in mesangial cells (Ishii et al., 1998). AR inhibitors have been investigated in experimental diabetic models and human DN, however, the results are inconsistent (Forbes et al., 2007). In the hexosamine pathway, glucose-6-phosphatase is converted to fructose-6-phophate as a part of the glycolytic pathway and subsequently converted to glucosamine-6-phosphate, and further uridine 5'-diphospho(UDP)-N-acetyl glucosamine (UDP-GlcNAc) by glutamine:fructose-6-phosphate aminotransferase (GFAT). UDP-GlcNAc provides O-GlcNAc modification of the serine/threonine residue of protein and competes with phosphorylation, leading to inhibition of the protein function such as insulin signaling (Yang et al., 2008). It has also been shown that the hexosamine pathway induces TGF-β synthesis (Weigert et al., 2001) and GFAT blockade inhibits the TGF-β expression as well as ECM production in mesangial cells (Schleicher and Weigert, 2000; Weigert et al., 2003).

Oxidative stress

Oxidative stress is induced by the imbalance of ROS generation and endogenous antioxidant activity. ROS

elicits inflammatory signaling pathways. There are numerous sources of ROS production, including NADPH oxidase and mitochondria. NADPH oxidase can be activated by an increase in the NADH/NAD$^+$ ratio induced by increased flux through the polyol pathway (Rask-Madsen and King, 2013). NADPH oxidase activity is increased by high-glucose and free fatty acid-mediated PKC activation (Inoguchi et al., 2000). AGEs have been shown to promote ROS generation by modulating catalytic sites in the molecular structure (Yagihashi, 1997) and via RAGE (Wautier et al., 2001). Mitochondria are an important source of ROS. Glucose influx into cells causes glycolysis to form pyruvate and then enters into the TCA cycle, leading to production of ATP through oxidative phosphorylation in the mitochondrial respiratory chain complex. NADH and FADH2 which are generated from the TCA cycle act as electron donors during oxidative phosphorylation to create a proton gradient over the inner mitochondrial membrane (Kashihara et al., 2010). In this process, NADH and FADH2 are transferred to molecular oxygen (O_2) and most of the O_2 is reduced to water; therefore, less than 1% of O_2 is converted to superoxide anion (Kashihara et al., 2010). Under mitochondrial dysfunction such as diabetes, the proton gradient increases and inhibits the transfer of electrons from coenzyme Q to complex III of the electron chain, thereby electrons are transferred to molecular oxygen, leading to excessive production of superoxide (Brownlee, 2001; Rask-Madsen and King, 2013).

NO has an antioxidant effect, but paradoxically, endothelial NO synthase (eNOS) can be a source of ROS by eNOS uncoupling. Tetrahydrobiopterin (BH_4) has been implicated in the mechanism of eNOS uncoupling (Li et al., 2014). To generate NO from L-arginine, eNOS requires cofactors such as BH_4. Under pathological conditions, including diabetes, BH_4 is oxidized to dihydrobiopterin (BH_2) and the amount of BH_4 is restricted, leading to production of superoxide but not NO (Li et al., 2014).

Rho/Rho-kinase

Rho is a small GTPase binding protein that belongs to the Ras superfamily. Rho is localized mainly in the cytosol as a GDP-bound form (inactive form). Under physiological conditions, Rho is translocated to the cell membrane, turns into the GTP-bound form (active form) and then activates downstream signaling through Rho-kinase, an effector of Rho (Ishizaki et al., 1996). Hyperglycemia, growth factors, and cytokines have been shown to activate Rho (Zhou et al., 2011b). Rho-kinase inhibits myosin phosphatase through myosin phosphatase subunit 1 (MYPT1) phosphorylation, thereby inducing cytoskeletal reorganization. Rho-kinase has two isoforms, ROCK1 and ROCK2. Increased Rho-kinase activity has been implicated in the pathogenesis of DN (Fig. 2). Rho activation is controlled by the three following factors. The first one is guanine exchange

factor (GEF). GEF promotes the conversion of GDP to GTP, thereby activating Rho. The second one is GTPase-activating protein (GAP), which promotes the conversion of GDP by hydrolyzing GTP and inhibits activation of Rho. The third one is guanine dissociation inhibitor (GDI), which suppresses the dissociation of GDP from Rho, maintaining Rho inactive. ROS and PKC are involved in high glucose-mediated Rho activation. It has been demonstrated that high glucose-mediated Rho-kinase activation can be inhibited by antioxidants in vascular endothelial cells (Rikitake and Liao, 2005). Since DAG promotes activation of GEF, the DAG-PKC pathway is thought to be involved in regulating Rho-kinase activity. In addition to PKCβ, the involvement of PKCα in regulating Rho activity has been suggested. PKCα phosphorylates GDI and inhibits its function, thereby inducing Rho activation (Mehta et al., 2001). Although more than 70 GEFs and GAPs have been identified, no specific GEF that is activated under diabetic conditions has been elucidated. cAMP/protein kinase A (PKA) is known to have an inhibitory effect on Rho. The endothelial expression of Rho/Rho-kinase is inhibited by GLP-1 receptor agonist exenatide, and this inhibition is negated by H89, a PKA inhibitor (Wang et al., 2013). Finally, statins have been shown to block Rho/Rho-kinase activity (Zhou et al., 2011b). To bind GTP, Rho undergoes isoprenylation (geranyl-geranylation). Statins are known to inhibit synthesis of isoprenoids (geranylgeranyl-pyrophosphate and farnesyl-pyrophosphate), intermediates of the cholesterol synthetic pathway, which results in suppression of Rho/Rho-kinase activity.

Rho/Rho-kinase in Diabetic Nephropathy

Mesangial cells

Excessive ECM production from mesangial cells is a major cause of glomerulosclerosis in DN. A number of factors are involved in this process, including growth factors, inflammatory cytokines, macrophage infiltration and hypoxia. Rho/Rho-kinase has been shown to regulate these factors, thereby promoting ECM production from mesangial cells. Our laboratory demonstrated that fasudil, a specific Rho-kinase inhibitor, ameliorated albuminuria in diabetic rats accompanied by inhibition of the TGF-β and Nox4 expression in the renal cortex (Gojo et al., 2007). A subsequent study confirmed that Rho-kinase inhibition attenuated albuminuria and pathological changes in the glomeruli, such as mesangial expansion and thickening of the glomerular basement membrane (GBM), in db/db mice (Peng et al., 2008). Accordingly, we investigated the mechanisms by which Rho-kinase promotes ECM production under diabetic conditions, focusing on transcription factors hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-ϰB that are involved in the pathogenesis of DN (Sanchez and Sharma, 2009; Isoe et al., 2010; Nayak et al., 2016). The administration of

fasudil attenuated albuminuria and glomerulosclerosis in db/db mice accompanied by inhibiting the expression levels of fibrotic factors such as TGF-β, CTGF, and fibronectin (Matoba et al., 2013). Nuclear translocation of HIF-1α was increased in the renal cortex of db/db mice. However, fasudil treatment attenuated the nuclear translocation of HIF-1α and the expression levels of HIF-1α target genes, such as VEGF and CTGF, in the renal cortex (Matoba et al., 2013). Rho-kinase blockade promoted proteasomal degradation of HIF-1α (Matoba et al., 2013), indicating that Rho-kinase prevents ubiquitination and subsequent proteasomal degradation of HIF-1α, thereby eliciting the fibrotic response in mesangial cells. We observed that Rho-kinase blockade inhibits macrophage infiltration into the glomeruli in db/db mice (Matoba et al., 2014). Moreover, TNF-α-mediated induction of chemokines, such as macrophage colony stimulating factor (M-CSF) and monocyte chemoattractant protein (MCP)-1, was inhibited by Rho-kinase blockade (Matoba et al., 2010; Matoba et al., 2014). A mechanistic analysis revealed that Rho-kinase blockade attenuates TNF-α-induced nuclear translocation of NF-κB by inhibiting p38MAPK-dependent

actin dynamics (Matoba et al., 2014). Taken together, Rho-kinase mediates nuclear translocation of HIF-1α and NF-κB under diabetic conditions, thereby promoting fibrotic and inflammatory responses in mesangial cells, leading to glomerulosclerosis.

Endothelial cells

Hyperpermeability of glomerular endothelial cells (GEnCs) plays an important role in the development of DN (Liu et al., 2007). The endothelial tight junction (TJ) is a structural barrier to maintain endothelial permeability. Occludin and zonula occludens (ZO)-1 are major components of the TJ complex. High glucose inhibits occludin expression and disrupts occludin/ZO-1 translocation, leading to hyperpermeability in GEnCs. Rho activation and subsequent actin cytoskeleton remodeling have been shown to be involved in VEGF-mediated hyperpermeability in GEnCs (Zeng et al., 2005). It has been shown that Rho/Rho-kinase activation mediates high glucose-induced dysregulation of occludin/ZO-1 in GEnCs. Furthermore, occludin/ZO-1 dysregulation is observed in the glomeruli of db/db mice
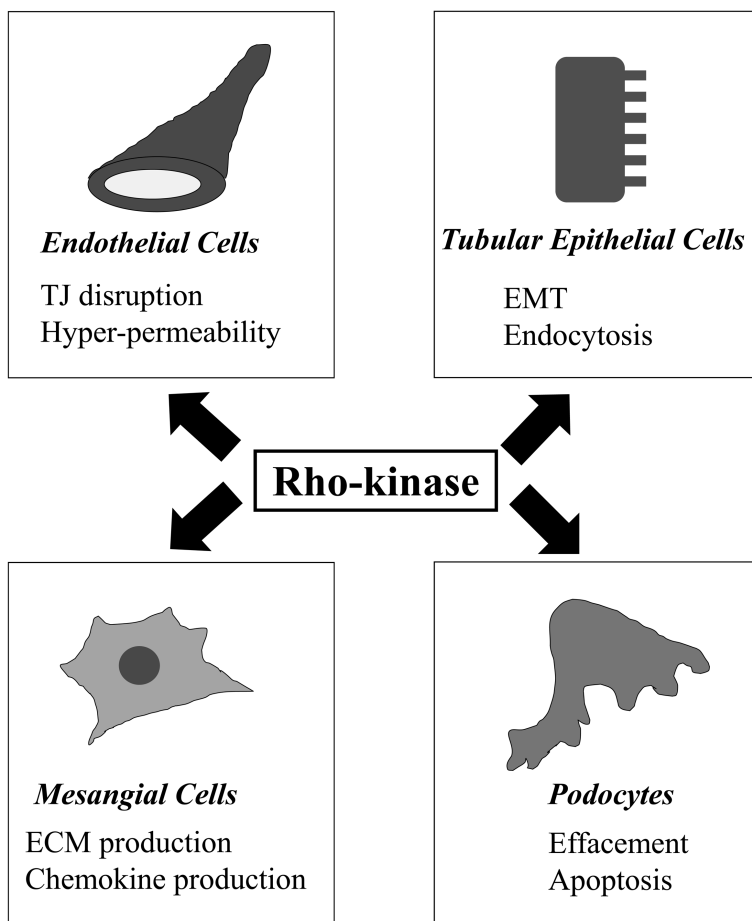

Endothelial Cells

TJ disruption
Hyper-permeability

Tubular Epithelial Cells

EMT
Endocytosis

Rho-kinase

Mesangial Cells

ECM production
Chemokine production

Podocytes

Effacement
Apoptosis

Fig. 2. Rho-kinase in DN. Rho-kinase activation disrupts the cellular function of mesangial cells, podocytes, glomerular endothelial cells, and tubular epithelial cells, leading to the development of DN.

accompanied by albuminuria and Rho/Rho-kinase activation, all of which are inhibited by statin (Peng et al., 2013). Recently, Rho-kinase has been shown to mediate high glucose-induced EndMT in GEnCs, leading to increased endothelial permeability (Peng et al., 2016). Interestingly, Rho-kinase inhibition suppressed α-SMA expression in GEnCs and albuminuria in db/db mice (Peng et al., 2016). These findings demonstrate that Rho-kinase plays an important role in the glomerular endothelial dysfunction and hyperpermeability that cause DN.

Podocytes

Podocyte abnormality is an important feature of DN. A previous study revealed that diabetic mouse models (STZ-induced diabetic mice and db/db mice) with targeted deletions of ROCK1 showed attenuation of albuminuria together with inhibition of glomerulosclerosis (Wang et al., 2012). An inducible podocyte-specific knock-in mouse expressing a constitutively active mutant of ROCK1 developed albuminuria and glomerulosclerosis in the diabetic milieu. ROCK1 was shown to mediate glomerular apoptosis and mitochondrial ROS production. Mechanistically, the authors identified ROCK1 as a potent regulator of mitochondrial dynamics by triggering mitochondrial fission by phosphorylating Drp1 at serine 600, a substrate of ROCK1, under diabetic conditions (Wang et al., 2012). Studies investigating the role of Rho in the podocyte function showed somewhat conflicting results. Welsh and colleagues reported that Rho has protective effects on podocytes. Rho activation by insulin signaling is essential for actin cytoskeleton remodeling in podocytes (Welsh et al., 2010). Asanuma et al. demonstrated that synaptopodin modulates the podocyte actin cytoskeleton and regulation of podocyte cell migration via regulating Rho (Asanuma et al., 2006). These studies suggest that Rho plays an important role in regulating the podocyte structure and function. Future studies are necessary to elucidate the precise role of Rho/Rho-kinase in podocytes.

Tubular epithelial cells

The epithelial-mesenchymal transition (EMT) of tubular epithelial cells is a crucial step in interstitial fibrosis. A number of studies have revealed that Rho/Rho-kinase signaling plays an important role in EMT. Disruption of TJ proteins such as occludin and ZO-1 is an early event in EMT (Blanco et al., 2004; Ozdamar et al., 2005). It has been shown that Rho-kinase inhibitor rescued TGF-β-mediated down-regulation of occludin and ZO-1 in human renal proximal tubular cells, indicating that Rho-kinase is involved in disruption of TJ proteins during EMT (Zhang et al., 2013). Furthermore, EMT has been shown to be attenuated by benidipine through inhibition of ROCK1, an isoform of Rho-kinase, accompanied by a decrease in the α-smooth muscle actin (SMA)

expression in diabetic rats (Wu et al., 2013). We also demonstrated that sphigosine-1-phosphate (S1P) induces Rho-kinase activation via its receptor S1P2, leading to EMT, such as changes in distribution of E-cadherin and an increase in the α-SMA expression in tubular epithelial cells (Ishizawa et al., 2014). These findings indicated that Rho-kinase plays an important role in the EMT of tubular epithelial cells.

The multiligand endocytic receptors megalin and cubilin have been shown to play a crucial role in protein reabsorption and albumin uptake in proximal tubular cells (Nielsen et al., 2016). A study utilizing ROCK1-deficient diabetic mice showed that decreased albuminuria was associated with protection against the loss of megalin and cubilin in the renal cortex (Zhou et al., 2011a). Consistent with this observation, Y-27632 rescued high glucose-mediated downregulation of megalin and cubilin in cultured tubular epithelial cells (Zhou et al., 2011a).

Conclusion

In this review, we highlighted various signaling pathways underlying DN. In addition to hemodynamic and metabolic mechanisms, a number of studies showed that Rho-kinase is a key factor in the development of DN. Rho-kinase may be an attractive therapeutic target against DN. To date, statin is a practical option for Rho-kinase inhibition. Future studies are required to validate the clinical application of Rho-kinase inhibitors against DN. Nevertheless, the most effective prevention for DN is to manage not only diabetes, but also hypertension and dyslipidemia because they all can trigger signaling pathways that are involved in the development of DN. In addition to such clinical efforts, future studies to identify the factor which governs these signaling pathways may provide a novel therapeutic target against DN.

References

Adler A.I., Stevens R.J., Manley S.E., Bilous R.W., Cull C.A. and Holman R.R. (2003). Development and progression of nephropathy in type 2 diabetes: The united kingdom prospective diabetes study (ukpds 64). Kidney Int. 63, 225-232.

Arendshorst W.J., Brannstrom K. and Ruan X. (1999). Actions of angiotensin ii on the renal microvasculature. J. Am. Soc. Nephrol. 10 (Suppl 11), S149-161.

Asanuma K., Yanagida-Asanuma E., Faul C., Tomino Y., Kim K. and Mundel P. (2006). Synaptopodin orchestrates actin organization and cell motility via regulation of rhoa signalling. Nat. Cell Biol. 8, 485-491.

Blanco D., Vicent S., Elizegi E., Pino I., Fraga M.F., Esteller M., Saffiotti U., Lecanda F. and Montuenga L.M. (2004). Altered expression of

adhesion molecules and epithelial-mesenchymal transition in silica-induced rat lung carcinogenesis. Lab. Invest. 84, 999-1012.

Brenner B.M., Cooper M.E., de Zeeuw D., Keane W.F., Mitch W.E., Parving H.H., Remuzzi G., Snapinn S.M., Zhang Z., Shahinfar S. and Investigators R.S. (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N. Engl. J. Med. 345, 861-869.

Brownlee M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813-820.

Carmines P.K., Ohishi K. and Ikenaga H. (1996). Functional impairment of renal afferent arteriolar voltage-gated calcium channels in rats with diabetes mellitus. J. Clin. Invest. 98, 2564-2571.

Checherita I.A., Manda G., Hinescu M.E., Peride I., Niculae A., Bilha S., Gramaticu A., Voroneanu L. and Covic A. (2016). New molecular insights in diabetic nephropathy. Int. Urol. Nephrol. 48, 373-387.

Cherney D.Z., Perkins B.A., Soleymanlou N., Maione M., Lai V., Lee A., Fagan N.M., Woerle H.J., Johansen O.E., Broedl U.C. and von Eynatten M. (2014). Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation 129, 587-597.

Craven P.A., Davidson C.M. and DeRubertis F.R. (1990). Increase in diacylglycerol mass in isolated glomeruli by glucose from de novo synthesis of glycerolipids. Diabetes 39, 667-674.

De Nicola L., Gabbai F.B., Liberti M.E., Sagliocca A., Conte G. and Minutolo R. (2014). Sodium/glucose cotransporter 2 inhibitors and prevention of diabetic nephropathy: Targeting the renal tubule in diabetes. Am. J. Kidney Dis. 64, 16-24.

Flyvbjerg A., Denner L., Schrijvers B.F., Tilton R.G., Mogensen T.H., Paludan S.R. and Rasch R. (2004). Long-term renal effects of a neutralizing rage antibody in obese type 2 diabetic mice. Diabetes 53, 166-172.

Forbes J.M., Fukami K. and Cooper M.E. (2007). Diabetic nephropathy: Where hemodynamics meets metabolism. Exp. Clin. Endocrinol. Diabetes 115, 69-84.

Forstermann U. and Sessa W.C. (2012). Nitric oxide synthases: Regulation and function. Eur. Heart J. 33, 829-837, 837a-837d.

Geraldes P. and King G.L. (2010). Activation of protein kinase c isoforms and its impact on diabetic complications. Circ. Res. 106, 1319-1331.

Gilbert R.E. (2014). Sodium-glucose linked transporter-2 inhibitors: Potential for renoprotection beyond blood glucose lowering? Kidney Int. 86, 693-700.

Gojo A., Utsunomiya K., Taniguchi K., Yokota T., Ishizawa S., Kanazawa Y., Kurata H. and Tajima N. (2007). The rho-kinase inhibitor, fasudil, attenuates diabetic nephropathy in streptozotocin-induced diabetic rats. Eur. J. Pharmacol. 568, 242-247.

Hayashi K., Epstein M., Loutzenhiser R. and Forster H. (1992). Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: Role of eicosanoid derangements. J. Am. Soc. Nephrol. 2, 1578-1586.

Helal I., Fick-Brosnahan G.M., Reed-Gitomer B. and Schrier R.W. (2012). Glomerular hyperfiltration: Definitions, mechanisms and clinical implications. Nat. Rev. Nephrol. 8, 293-300.

Hofmann M.A., Drury S., Fu C., Qu W., Taguchi A., Lu Y., Avila C., Kambham N., Bierhaus A., Nawroth P., Neurath M.F., Slattery T., Beach D., McClary J., Nagashima M., Morser J., Stern D. and Schmidt A.M. (1999). Rage mediates a novel proinflammatory axis: A central cell surface receptor for s100/calgranulin polypeptides. Cell 97, 889-901.

Inoguchi T., Li P., Umeda F., Yu H.Y., Kakimoto M., Imamura M., Aoki T., Etoh T., Hashimoto T., Naruse M., Sano H., Utsumi H. and Nawata H. (2000). High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase c-dependent activation of nad(p)h oxidase in cultured vascular cells. Diabetes 49, 1939-1945.

Ishii H., Jirousek M.R., Koya D., Takagi C., Xia P., Clermont A., Bursell S.E., Kern T.S., Ballas L.M., Heath W.F., Stramm L.E., Feener E.P. and King G.L. (1996). Amelioration of vascular dysfunctions in diabetic rats by an oral pkc beta inhibitor. Science 272, 728-731.

Ishii H., Tada H. and Isogai S. (1998). An aldose reductase inhibitor prevents glucose-induced increase in transforming growth factor-beta and protein kinase c activity in cultured mesangial cells. Diabetologia 41, 362-364.

Ishizaki T., Maekawa M., Fujisawa K., Okawa K., Iwamatsu A., Fujita A., Watanabe N., Saito Y., Kakizuka A., Morii N. and Narumiya S. (1996). The small gtp-binding protein rho binds to and activates a 160 kda ser/thr protein kinase homologous to myotonic dystrophy kinase. EMBO J. 15, 1885-1893.

Ishizawa S., Takahashi-Fujigasaki J., Kanazawa Y., Matoba K., Kawanami D., Yokota T., Iwamoto T., Tajima N., Manome Y. and Utsunomiya K. (2014). Sphingosine-1-phosphate induces differentiation of cultured renal tubular epithelial cells under rho kinase activation via the s1p2 receptor. Clin. Exp. Nephrol. 18, 844-852.

Isoe T., Makino Y., Mizumoto K., Sakagami H., Fujita Y., Honjo J., Takiyama Y., Itoh H. and Haneda M. (2010). High glucose activates HIF-1-mediated signal transduction in glomerular mesangial cells through a carbohydrate response element binding protein. Kidney Int. 78, 48-59.

Jensen L.J., Denner L., Schrijvers B.F., Tilton R.G., Rasch R. and Flyvbjerg A. (2006). Renal effects of a neutralising rage-antibody in long-term streptozotocin-diabetic mice. J. Endocrinol. 188, 493-501.

Kagami S., Border W.A., Miller D.E. and Noble N.A. (1994). Angiotensin ii stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. J. Clin. Invest. 93, 2431-2437.

Kashihara N., Haruna Y., Kondeti V.K. and Kanwar Y.S. (2010). Oxidative stress in diabetic nephropathy. Curr. Med. Chem. 17, 4256-4269.

Koya D., Haneda M., Nakagawa H., Isshiki K., Sato H., Maeda S., Sugimoto T., Yasuda H., Kashiwagi A., Ways D.K., King G.L. and Kikkawa R. (2000). Amelioration of accelerated diabetic mesangial expansion by treatment with a pkc beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. FASEB J. 14, 439-447.

Li H., Horke S. and Forstermann U. (2014). Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 237, 208-219.

Liu E., Morimoto M., Kitajima S., Koike T., Yu Y., Shiiki H., Nagata M., Watanabe T. and Fan J. (2007). Increased expression of vascular endothelial growth factor in kidney leads to progressive impairment of glomerular functions. J. Am. Soc. Nephrol. 18, 2094-2104.

Lu M., Kuroki M., Amano S., Tolentino M., Keough K., Kim I., Bucala R. and Adamis A.P. (1998). Advanced glycation end products increase retinal vascular endothelial growth factor expression. J. Clin. Invest. 101, 1219-1224.

Makino H., Haneda M., Babazono T., Moriya T., Ito S., Iwamoto Y., Kawamori R., Takeuchi M., Katayama S. and Group I.S. (2007). Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes. Diabetes Care 30, 1577-

1578.

Matoba K., Kawanami D., Ishizawa S., Kanazawa Y., Yokota T. and Utsunomiya K. (2010). Rho-kinase mediates tnf-alpha-induced mcp-1 expression via p38 mapk signaling pathway in mesangial cells. Biochem. Biophys. Res. Commun. 402, 725-730.

Matoba K., Kawanami D., Okada R., Tsukamoto M., Kinoshita J., Ito T., Ishizawa S., Kanazawa Y., Yokota T., Murai N., Matsufuji S., Takahashi-Fujigasaki J. and Utsunomiya K. (2013). Rho-kinase inhibition prevents the progression of diabetic nephropathy by downregulating hypoxia-inducible factor 1alpha. Kidney Int. 84, 545-554.

Matoba K., Kawanami D., Tsukamoto M., Kinoshita J., Ito T., Ishizawa S., Kanazawa Y., Yokota T., Murai N., Matsufuji S. and Utsunomiya K. (2014). Rho-kinase regulation of tnf-alpha-induced nuclear translocation of nf-kappab rela/p65 and m-csf expression via p38 mapk in mesangial cells. Am. J. Physiol. Renal. Physiol. 307, F571-580.

Mehta D., Rahman A. and Malik A.B. (2001). Protein kinase c-alpha signals rho-guanine nucleotide dissociation inhibitor phosphorylation and rho activation and regulates the endothelial cell barrier function. J. Biol. Chem. 276, 22614-22620.

Menne J., Shushakova N., Bartels J., Kiyan Y., Laudeley R., Haller H., Park J.K. and Meier M. (2013). Dual inhibition of classical protein kinase c-alpha and protein kinase c-beta isoforms protects against experimental murine diabetic nephropathy. Diabetes 62, 1167-1174.

Menne J., Park J.K., Boehne M., Elger M., Lindschau C., Kirsch T., Meier M., Gueler F., Fiebeler A., Bahlmann F.H., Leitges M. and Haller H. (2004). Diminished loss of proteoglycans and lack of albuminuria in protein kinase c-alpha-deficient diabetic mice. Diabetes 53, 2101-2109.

Mima A., Kitada M., Geraldes P., Li Q., Matsumoto M., Mizutani K., Qi W., Li C., Leitges M., Rask-Madsen C. and King G.L. (2012). Glomerular vegf resistance induced by pkcdelta/shp-1 activation and contribution to diabetic nephropathy. FASEB J. 26, 2963-2974.

Nagahama T., Hayashi K., Ozawa Y., Takenaka T. and Saruta T. (2000). Role of protein kinase c in angiotensin ii-induced constriction of renal microvessels. Kidney Int. 57, 215-223.

Nayak B.K., Shanmugasundaram K., Friedrichs W.E., Cavaglierii R.C., Patel M., Barnes J. and Block K. (2016). Hif-1 mediates renal fibrosis in ove26 type 1 diabetic mice. Diabetes (in press).

Newton A.C. (2001). Protein kinase C: Structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem. Rev. 101, 2353-2364.

Nielsen R., Christensen E.I. and Birn H. (2016). Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. Kidney Int. 89, 58-67.

Ohshiro Y., Ma R.C., Yasuda Y., Hiraoka-Yamamoto J., Clermont A.C., Isshiki K., Yagi K., Arikawa E., Kern T.S. and King G.L. (2006). Reduction of diabetes-induced oxidative stress, fibrotic cytokine expression, and renal dysfunction in protein kinase cbeta-null mice. Diabetes 55, 3112-3120.

Ozdamar B., Bose R., Barrios-Rodiles M., Wang H.R., Zhang Y. and Wrana J.L. (2005). Regulation of the polarity protein par6 by tgfbeta receptors controls epithelial cell plasticity. Science 307, 1603-1609.

Peng F., Wu D., Gao B., Ingram A.J., Zhang B., Chorneyko K., McKenzie R. and Krepinsky J.C. (2008). Rhoa/rho-kinase contribute to the pathogenesis of diabetic renal disease. Diabetes 57, 1683-1692.

Peng H., Luo P., Li Y., Wang C., Liu X., Ye Z., Li C. and Lou T. (2013).

Simvastatin alleviates hyperpermeability of glomerular endothelial cells in early-stage diabetic nephropathy by inhibition of rhoa/rock1. PLoS One 8, e80009.

Peng H., Li Y., Wang C., Zhang J., Chen Y., Chen W., Cao J., Wang Y., Hu Z. and Lou T. (2016). Rock1 induces endothelial-to-mesenchymal transition in glomeruli to aggravate albuminuria in diabetic nephropathy. Sci. Rep. 6, 20304.

Premaratne E., Verma S., Ekinci E.I., Theverkalam G., Jerums G. and MacIsaac R.J. (2015). The impact of hyperfiltration on the diabetic kidney. Diabetes Metab. 41, 5-17.

Ramasamy R., Yan S.F. and Schmidt A.M. (2011). Receptor for age (rage): Signaling mechanisms in the pathogenesis of diabetes and its complications. Ann. NY Acad. Sci. 1243, 88-102.

Rask-Madsen C. and King G.L. (2013). Vascular complications of diabetes: Mechanisms of injury and protective factors. Cell. Metab. 17, 20-33.

Reiniger N., Lau K., McCalla D., Eby B., Cheng B., Lu Y., Qu W., Quadri N., Ananthakrishnan R., Furmansky M., Rosario R., Song F., Rai V., Weinberg A., Friedman R., Ramasamy R., D'Agati V. and Schmidt A.M. (2010). Deletion of the receptor for advanced glycation end products reduces glomerulosclerosis and preserves renal function in the diabetic ove26 mouse. Diabetes 59, 2043-2054.

Rikitake Y. and Liao J.K. (2005). Rho-kinase mediates hyperglycemia-induced plasminogen activator inhibitor-1 expression in vascular endothelial cells. Circulation 111, 3261-3268.

Ruiz-Ortega M., Lorenzo O., Ruperez M., Konig S., Wittig B. and Egido J. (2000). Angiotensin ii activates nuclear transcription factor kappab through at(1) and at(2) in vascular smooth muscle cells: Molecular mechanisms. Circ Res 86, 1266-1272.

Sanchez A.P. and Sharma K. (2009). Transcription factors in the pathogenesis of diabetic nephropathy. Expert. Rev. Mol. Med. 11, e13.

Schleicher E.D. and Weigert C. (2000). Role of the hexosamine biosynthetic pathway in diabetic nephropathy. Kidney Int. (Suppl 77), S13-18.

Scivittaro V., Ganz M.B. and Weiss M.F. (2000). Ages induce oxidative stress and activate protein kinase c-beta(ii) in neonatal mesangial cells. Am. J. Physiol. Renal. Physiol. 278, F676-683.

Taguchi A., Blood D.C., del Toro G., Canet A., Lee D.C., Qu W., Tanji N., Lu Y., Lalla E., Fu C., Hofmann M.A., Kislinger T., Ingram M., Lu A., Tanaka H., Hori O., Ogawa S., Stern D.M. and Schmidt A.M. (2000). Blockade of rage-amphoterin signalling suppresses tumour growth and metastases. Nature 405, 354-360.

Tikellis C., Brown R., Head G.A., Cooper M.E. and Thomas M.C. (2014). Angiotensin-converting enzyme 2 mediates hyperfiltration associated with diabetes. Am. J. Physiol. Renal. Physiol. 306, F773-780.

Vlassara H., Striker L.J., Teichberg S., Fuh H., Li Y.M. and Steffes M. (1994). Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. Proc. Natl. Acad. Sci. USA 91, 11704-11708.

Wang D., Luo P., Wang Y., Li W., Wang C., Sun D., Zhang R., Su T., Ma X., Zeng C., Wang H., Ren J. and Cao F. (2013). Glucagon-like peptide-1 protects against cardiac microvascular injury in diabetes via a camp/pka/rho-dependent mechanism. Diabetes 62, 1697-1708.

Wang W., Wang Y., Long J., Wang J., Haudek S.B., Overbeek P., Chang B.H., Schumacker P.T. and Danesh F.R. (2012). Mitochondrial fission triggered by hyperglycemia is mediated by

rock1 activation in podocytes and endothelial cells. Cell Metab. 15, 186-200.

Wautier M.P., Chappey O., Corda S., Stern D.M., Schmidt A.M. and Wautier J.L. (2001). Activation of nadph oxidase by age links oxidant stress to altered gene expression via rage. Am. J. Physiol. Endocrinol. Metab. 280, E685-694.

Weigert C., Brodbeck K., Lehmann R., Haring H.U. and Schleicher E.D. (2001). Overexpression of glutamine:Fructose-6-phosphate-amidotransferase induces transforming growth factor-beta1 synthesis in nih-3t3 fibroblasts. FEBS Lett. 488, 95-99.

Weigert C., Friess U., Brodbeck K., Haring H.U. and Schleicher E.D. (2003). Glutamine:Fructose-6-phosphate aminotransferase enzyme activity is necessary for the induction of tgf-beta1 and fibronectin expression in mesangial cells. Diabetologia 46, 852-855.

Welsh G.I., Hale L.J., Eremina V., Jeansson M., Maezawa Y., Lennon R., Pons D.A., Owen R.J., Satchell S.C., Miles M.J., Caunt C.J., McArdle C.A., Pavenstadt H., Tavare J.M., Herzenberg A.M., Kahn C.R., Mathieson P.W., Quaggin S.E., Saleem M.A. and Coward R.J. (2010). Insulin signaling to the glomerular podocyte is critical for normal kidney function. Cell Metab. 12, 329-340.

Wendt T.M., Tanji N., Guo J., Kislinger T.R., Qu W., Lu Y., Bucciarelli L.G., Rong L.L., Moser B., Markowitz G.S., Stein G., Bierhaus A., Liliensiek B., Arnold B., Nawroth P.P., Stern D.M., D'Agati V.D. and Schmidt A.M. (2003). Rage drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. Am. J. Pathol. 162, 1123-1137.

Wu G., Xu M., Xu K. and Hu Y. (2013). Benidipine protects kidney through inhibiting rock1 activity and reducing the epithelium-mesenchymal transdifferentiation in type 1 diabetic rats. J. Diabetes Res. 2013, 174526.

Xia P., Inoguchi T., Kern T.S., Engerman R.L., Oates P.J. and King G.L. (1994). Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase c pathway in diabetes and hypergalactosemia. Diabetes 43, 1122-1129.

Yagihashi S. (1997). Pathogenetic mechanisms of diabetic neuropathy: Lessons from animal models. J. Peripher. Nerv. Syst. 2, 113-132.

Yale J.F., Bakris G., Cariou B., Nieto J., David-Neto E., Yue D., Wajs E., Figueroa K., Jiang J., Law G., Usiskin K., Meininger G. and Group D.I.A.S. (2014). Efficacy and safety of canagliflozin over 52 weeks in patients with type 2 diabetes mellitus and chronic kidney disease. Diabetes Obes. Metab. 16, 1016-1027.

Yang X., Ongusaha P.P., Miles P.D., Havstad J.C., Zhang F., So W.V., Kudlow J.E., Michell R.H., Olefsky J.M., Field S.J. and Evans R.M. (2008). Phosphoinositide signalling links o-glcnac transferase to insulin resistance. Nature 451, 964-969.

Zeng L., Xu H., Chew T.L., Eng E., Sadeghi M.M., Adler S., Kanwar Y.S. and Danesh F.R. (2005). HMG CoA reductase inhibition modulates VEGf-induced endothelial cell hyperpermeability by preventing rhoa activation and myosin regulatory light chain phosphorylation. FASEB J. 19, 1845-1847.

Zhang K., Zhang H., Xiang H., Liu J., Liu Y., Zhang X., Wang J. and Tang Y. (2013). Tgf-beta1 induces the dissolution of tight junctions in human renal proximal tubular cells: Role of the rhoa/rock signaling pathway. Int. J. Mol. Med. 32, 464-468.

Zhou L., Liu F., Huang X.R., Chen H., Chung A.C., Shi J., Wei L., Lan H.Y. and Fu P. (2011a). Amelioration of albuminuria in rock1 knockout mice with streptozotocin-induced diabetic kidney disease. Am. J. Nephrol. 34, 468-475.

Zhou Q., Gensch C. and Liao J.K. (2011b). Rho-associated coiled-coil-forming kinases (rocks): Potential targets for the treatment of atherosclerosis and vascular disease. Trends. Pharmacol. Sci. 32, 167-173.